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Supplemental Material

Chronic Exposure to Low Doses of Dioxin Promotes Liver Fibrosis Development in the C57BL6/J Diet-Induced Obesity Mouse Model

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Figure S1. Distribution of potential low and high gainer individuals in the "TCDD" and "control" subgroups at week 5 of the LFD/HFD intervention. After mice received either a LFD or a HFD for 5 weeks (n=30), body weight (BW) gain and plasma leptin levels were determined, as described in the Methods, to randomize the mice into two subgroups for future injections (from week 8) with 5µg/kg of TCDD (LF-tcdd, n=16 and HF-tcdd, n=16, respectively) or the vehicle (LF-ctrl, n=14 and HF-ctrl, n=14, respectively). A. Variability in BW gain and plasma leptin concentrations at week 5 within the four different subgroups of injection. B. Potential low and high "gainer" status of the mice based on body weight gain and leptin level at week 5 (see also Table S1). In the HFD group, a BW gain of 5 g (and over) and a plasma leptin level of 5 ng/mL (and over) (red lines) were chosen to discriminate potential high gainers (zone 1) and low gainers (zone 2). In the LFD group, low and high gainers do not differentiate yet at week 5, based on those parameters (zone 2). However, the mice with barely detectable leptin levels or with a level of 2.5 ng/mL (and over) might be considered as potential low or high gainers, respectively. All the animals were divided equally in the control and TCDD subgroups. As mentioned in the Methods, two animals were excluded from the study at the time of sacrifice (animals 261 and 227), which belonged respectively to the LF-ctrl and LF-tcdd groups. Data are expressed as mean±SEM; a, versus LF-ctrl; b, versus LF-tcdd; p<0.001.

Figure S2. Effect of increasing doses of TCDD on hepatic parameters and on the hepatic mRNA levels of selected genes. Mice were injected with different doses of TCDD (0.1, 1, 10, 25 µg/kg) or the vehicle (Ctrl) during 6 weeks. A. The levels of hepatic *Cyp1a1* mRNA were measured by qPCR (see below). B. Liver weight (LW) as a % of body weight (BW) of the mice. C. The hepatic triglyceride (TG) content. D. Alanine aminotransferase (ALAT) activity in the serum. E. Histological stainings of representative liver sections of the various treatments: hematoxylin-eosin staining (top panel), with red and black

arrowheads indicating lipid droplets and infiltrated inflammatory cells, respectively and picro-sirius red staining (bottom panel), with large black arrows pointing to fibrotic scars of collagen I and III (bar = 100 μ m). The hepatic mRNA levels of marker genes for (F) lipid metabolism (*Cd36*, *Pparg*, *Plin2/Adrp* and *Dgat2*), (G) inflammation (*Cd68*) and (H) fibrosis (*Tgfb1*, *Acta2* and *Colla1*) were measured by qPCR. The relative mRNA levels were estimated using the delta-delta Ct method with *Gapdh* as the reference. Mean expression in the ctrl group is set at 100%. Data are expressed as mean \pm SEM; *, p<0.05; **, p<0.01; ***, p<0.001; as compared to ctrl; \$, p<0.05; \$\$, p<0.01; as compared to TCDD0.1.

Figure S3. Physiologically-based pharmacokinetic modeling of the levels of TCDD in the organs of mice after intra-peritoneal injections of 5 μ g/kg TCDD for 6 weeks. A computerized model was used to estimate the levels of TCDD in the organs of mice following injections with 5 μ g/kg TCDD. The time is indicated in hours and the TCDD concentration in ppt. The final TCDD concentrations in ppt wet weight are 32,490 ppt for the adipose tissue (blue curve), 56,834 ppt for the liver (green curve) and 66.8 ppt for blood (red curve).